Eulachon Embryonic Egg and Larval Outdrift Sampling Manual for Ocean and River Surveys

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by

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ABSTRACT

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The anadromous eulachon (*Thaleichthys pacificus*) spawns in the lower reaches of coastal rivers and streams from northern California to Alaska. Although the distribution and timing in some rivers is well known, the occurrence in other rivers is uncertain or unknown. The presence of larval eulachon in estuaries and marine waters adjacent to rivers is a strong indication that a river is used by eulachon for spawning. Ichthyoplankton surveys designed to detect the presence or absence of eulachon larvae in coastal estuaries and inlets are easily conducted with minimal expense. Survey timing is the most important consideration. Eulachon spawning stock biomass (SSB) estimations based on in-river, egg and larval density measurements, however, require intensive replicate sampling to insure adequate spatial and temporal coverage. A swept volume (n/m^3), bongo net technique and daily river discharge measurements (m^3/s) are used to estimate egg and larval production. Accurate fecundity determinations of spawning adults are also required in the calculation. In this manual, we describe and discuss the equipment and techniques that have been applied to estimate eulachon spawning stock biomass in the lower Fraser River and eulachon larval distribution and dispersion in central British Columbia mainland inlets and estuaries.

RÉSUMÉ

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L'eulakane (*Thaleichthys pacificus*) anadrome fraye dans le cours inférieur des fleuves et ruisseaux côtiers, du nord de la Californie à l'Alaska. Si sa répartition et son calendrier d'occupation de certaines rivières sont bien connus, son occurrence dans d'autres cours d'eau est inconnue ou incertaine. La présence de larves d'eulakane dans les estuaires et les eaux marines proches d'une embouchure est une bonne indication que le cours d'eau est fréquenté par ce poisson pour la fraye. Les relevés de l'ichthyoplancton conçus pour déterminer la présence ou l'absence de larves d'eulakane dans les estuaires et les bras de mer sont faciles à organiser et peu coûteux. L'aspect le plus important est le calendrier des relevés. Par contre, les estimations de la biomasse reproductrice à partir de la densité des œufs et des larves dans les cours d'eau nécessitent un échantillonnage intensif et répété pour que la couverture spatiale et temporelle soit satisfaisante. Nous nous servons du volume balayé (n/m^3), de la technique de prélèvement au filet bongo et de mesures du débit quotidien du cours d'eau (m^3/s) pour estimer la production d'œufs et de larves. Il est aussi nécessaire de disposer d'estimations correctes de la fécondité des adultes géniteurs pour les intégrer aux calculs. Dans ce manuel, nous décrivons et analysons le matériel et les techniques qui ont été employés pour estimer la biomasse reproductrice de l'eulakane dans le cours inférieur du Fraser River ainsi que la répartition et la dispersion des larves de ce poisson dans les bras de mer et les estuaires du centre de la côte continentale de la Colombie-Britannique.

INTRODUCTION

Ichthyoplankton surveys have being conducted for many years by various organizations. Sampling protocols have been developed and adapted for specific research studies or areas of interest. This manual was written to assist those specifically interested in assessing eulachon-bearing rivers, streams, estuaries and nearshore waters of British Columbia (BC). The techniques described here are general in nature and not specific to any particular river or ocean environment. Most procedures described in this manual are based on extensive sampling of larval rearing areas of Pacific herring (*Clupea pallasi*), as well as, the anadromous eulachon (*Thaleichthys pacificus*) in the nearshore waters and estuaries of BC. These techniques have been adapted from standard ichthyoplankton sampling procedures used extensively in many parts of the world.

Most larval fish surveys conducted in BC were done as a means to comment on larval dispersion and ecology (Stevenson 1962; Barraclough 1967; Rees 1969 and 1970, McGurk 1989; Crawford *et al.* 1990). Elsewhere, larval fish surveys are routinely used as a means of estimating stock abundance (Smith and Richardson 1977). Hay and McCarter (1991, 1997) and McCarter and Hay (1999) have conducted synoptic ocean surveys of Pacific herring and eulachon larvae to comment on stock structure. Recently, Hay *et al.* (1997, 2002) have adapted standard ichthyoplankton sampling techniques to assess eulachon spawning stock biomass in rivers. This manual emphasizes equipment, procedures and practises, citing specific examples of larval fish surveys conducted between 1985 and 2002 in British Columbia.

GENERAL SURVEY DESIGN

OCEAN SURVEYS

Ichthyoplankton sampling sites should be arranged to examine the waters adjacent to eulachon spawning areas (Fig. 1) as identified by Hay *et al.* (1997) and Hay and McCarter (2000). Sampling stations for ocean surveys of 2 to 4 week duration should be distributed at approximately 4 to 6 km intervals along various waterways in order to cover many of BC's inlets, channels and deep fjords. A zigzag pattern crossing both sides of an inlet or channel is sometimes necessary. A grid pattern may be traversed to cover larger bodies of water such as a sound or strait. A relatively large survey area may be required since eulachon larvae may be well dispersed along the BC coast by mid-April, May or June. Fixed distance, sampling intervals can simplify area extrapolation procedures that may be used to calculate indices of abundance. Consistent geographic spacing of plankton net towing sites also provides a set time to wash down plankton nets and preserve samples in 5% formalin while the research vessel is travelling at 9 to 10 knots to the next sampling station. Between 16 and 24 samples (approximately one tow every 20 to 30 minutes) can be collected in an 8-hour day using this design over a 10 to 12 consecutive day period. Evening hours can be spent travelling to new survey areas. Whalers or inflatable craft can also be deployed to sample nearshore stations (or stations off the main survey route) as greater speed and manoeuvrability permits accessing difficult-to-reach, larval fish dispersal areas. Slower, larger vessels and smaller, faster vessels will cover about the same number of stations in a day because the time gained by a smaller, faster vessel is lost by the additional time required to

remain on station while nets are being washed down and plankton samples preserved. A research vessel with two auxiliary whalers can collectively conduct between 48 and 72 sampling tows per day (at 5 km intervals) over a two week period. Eulachon larvae can be readily captured with plankton net gear in most BC mainland inlets and channels between mid-April and August. Large or small scale surveys can be considered, however, a survey design must always consider anticipated travelling time, fuel consumption, nautical and sampling equipment reliability, contingency plans, suitable accommodations, communications and safety.

RIVER SURVEYS

River sampling stations should be established at sites where vessel traffic and accessibility problems are not a major deterrent (e.g. swing bridges, exposed weather areas, opposing ebb tides). In the lower Fraser River we have concentrated on several sampling sites covering the North and South Arms of the Fraser River and the lower Pitt River area. Inter-annual changes in eulachon spawning distributions should also be considered when establishing sampling sites. In the past we have completed as many as 18 sites, sampling as far upriver as Mission and Hatzic Slough. Time restraints, however, often confine intensive sampling to 4 major sites (Deas Island, Tilbury Island, New Westminster and Barnston Island) and 3 minor sites (Iona Island on the lower North Arm, the upper North Arm and the lower Pitt River area). At each site, plankton net tows have been conducted approximately mid-river as well as near both banks of the river (i.e. 3 position, cross-river transect). We have confined most plankton net tows to daylight hours for safety and logistical reasons even though there is evidence that many larvae hatch at night. The spawning and incubation period of eulachon in the Fraser River is sufficiently protracted to cause gradual downstream dispersal of progeny into the sampling areas. Bi-weekly sampling of eggs and larvae at strategic sites has shown to be sufficient in delineating unimodal, egg and larval outdrift monitoring curves that differ significantly only by the timing and magnitude of the runs (Hay *et al.* 2002). Sudden pulses of eggs and larvae in smaller rivers, however, may disperse more rapidly, requiring daily or more frequent sampling schedules. The major consideration in any survey design is insuring adequate spatial and temporal sampling coverage, fully utilizing available time and resources. Eulachon embryonic egg and larval outdrift in the Fraser River usually begins during the last week in April, peaks during the last two weeks of May and declines by mid-June. Bi-weekly sampling of several sampling sites, positions and depths over an 6 to 8 week period is necessary to cover the main pulse of embryonic egg and larval outdrift. We have typically collected between 350 and 650, 1 litre jar samples over a 7 week period. Sampling coverage can be reduced as the patterns of several years of data are established and "key" representative sampling sites are identified.

ICHTHYOPLANKTON SAMPLING GEAR AND PROCEDURES

Ichthyoplankton samples can be collected using 57 cm diameter bongo nets hauled from large vessels or with smaller, 19 cm diameter bongo nets hauled from small or large vessels. The paired plankton nets on our 57 cm and 19 cm bongo frames are 3 metres and one metre in length respectively and are constructed of 0.35 mm aperture black, nylon Nitex® mesh. The Nitex® material, manufactured in Switzerland can be ordered from B. & S. H. Thompson Co. Ltd., Montreal, Canada, or Tetko, Inc., New York, USA and the nets can be constructed locally or

custom specifications can be accommodated by some oceanographic equipment suppliers (Fig. 2). The net designs we have chosen provide a bag length-to-mouth ratio of 5:1 for both sizes of bongo nets. A wide, 12 cm dacron or nylon "bag cloth" collar at the mouth and cod-end of the nets reduces excessive wear from fouling on deployment or handling on deck and also provides a durable surface for several stainless steel, hose clamps (joined together) to grip the nets to the bongo frame. The fabric of each net should be regularly inspected for wear holes and immediately replaced or mended as required. Plankton net mesh apertures smaller than 0.35 mm or larger than 0.6 mm in size are generally not recommended for eulachon egg and larval surveys. Eulachon eggs are 0.8 to 1.0 mm in diameter and the heads of newly hatched larvae are 0.6 to 0.8 mm in size. Net mesh apertures larger than 0.6 mm may extrude some eggs and larvae. At the lower end of the mesh size range, a plankton net's water filtering performance can be reduced if apertures are less than 0.35 mm in size, as meshes can become rapidly blocked when passing through plankton blooms (fibrous or filamentous material). A pressure wave can develop in front of a plankton net under tow and deflect fish larvae away from the net opening. Continuous net washing becomes necessary to clear obstructed meshes. Furthermore, fine-mesh plankton nets are not as durable as coarser nets. Fine-mesh nets (with mesh apertures less than 0.35 mm in size) require a finer monofilament fibre in their construction and do not withstand the rigors of intensive field use to the same extent as coarser nets.

General Oceanic Inc.® (Miami, Florida) digital, flow meters with low-speed (16.5 cm diameter) or high-speed (7.0 cm diameter) rotors may be used to measure the volume of water filtered through large or small bongo nets respectively. Flow meter interiors should be well maintained by flushing and rinsing with clean tap water after every survey and air-dried to prevent corrosion and grit accumulation. Unusually low flow meter readings may indicate a problem with the gears, shaft or rotor and may require replacement. Flow meters are tied into the center opening of the nets and positioned in the direction of flow so that the flow meter rotor can rotate freely and the number of revolutions can easily be recorded after each bongo net tow. In high wind conditions it may become necessary to use tethered, rotor clips to prevent the rotors from excessively turning while the net is entering or exiting the water. Plankton net tows are normally completed in 6 minutes while the towing vessel travels at approximately 3.5 km/hr or about 2 knots. Our chosen tow duration and net opening diameter provides a density measuring resolution limit of approximately 1 larvae per 100 m^3 of seawater filtered through a 57 cm diameter bongo net and 1 larvae per 20 m^3 of seawater through a 19 cm diameter net or $\pm$ 0.01 to 0.05 larvae/m^3.

The 57 cm diameter bongo nets with an 86 kg weight and 25 m of marked cable are slowly paid out from a research vessel at a rate of approximately 1 m every 7 or 8 seconds and immediately recovered at the same rate. This type of plankton net deployment is known as a standard, oblique tow. A slow descent and ascent rate facilitates intensive sampling of the top 20 metres of the water column where most fish larvae are captured. A maximum vertical net depth of 20 m is estimated based on a typical 30 to 40 $^{\circ}$ wire angle. The bongo nets are towed from an oceanographic A-frame (Fig. 3) and towing block using a 3/8 or 1/2 inch wire rope cable and a bronze, ball bearing swivel to ease deployment and reduce the chance of fouling. A heavy 86 kg weight is shackled immediately below the bongo frame yoke as considerable net drag can increase the wire angle making it difficult to keep the gear at the required towing depth especially when the nets are towed against strong tidal currents. The bongo weight should have a broad, flat base for immediate stability when the gear is recovered onto pitching boat decks. The large

bongo frames are constructed of two 0.5 cm plate aluminium circles 30 cm long, joined by a steel swivel yoke and painted with black anti-corrosion paint.

The smaller, 19 cm diameter bongo nets with a 15 kg weight and 20 m of marked cable are paid out from 5 to 6 metre whalers at a rate of 0.33 m/sec and immediately raised 1 m every 15 seconds. This type of tow is known as stepped, oblique tow. Each whaler is equipped with a small, single speed (0.33 m/sec) electric winch with a 3/16 inch diameter stainless steel wire cable, trolling blocks and aluminium towing gantry. As only one winch speed is possible, the nets are raised in a stepped fashion in order to maintain a consistent 6 minute towing duration. The smaller bongo frames are constructed of two, 19 cm inside diameter PVC (Polyvinyl chloride) pipes, 30 cm long joined by a steel swivel yoke and painted with black anti-corrosion paint. An oceanographic meter block (accommodating 3/16 inch wire) can be used for deeper tows to avoid the necessity of marking the wire cable at 1 metre intervals. This very portable and rugged bongo gear can be easily adapted to accommodate most vessels and situations. An aluminium boom and snatch block, for example, can be rigged to provide a temporary davit to tow the bongo nets using the net drum of a standard gillnet vessel.

SCOR plankton nets or other types of high efficiency hoop nets, equipped with digital flow meters, can also be towed from small inflatable boats or whalers in order to access nearshore stations or stations not on the main survey route. SCOR nets do not have a heavy bongo frame but consist of light, stainless steel, 60 cm diameter, double hoops separating the cylindrical and conical portions of the net and are towed from a swivel and a 3 point, stainless steel wire bridle. SCOR nets are more portable than bongo nets, but the nets do not afford an unobstructed opening and only single samples can be collected per tow. Also, more care must be taken with hoop nets to avoid sea or river bottom shaffing, dredging or fouling. A small, 10 to 15 kg cannon ball is shackled to the SCOR net so that the light hoop net can often be hauled by hand or by hand winch. SCOR nets can be towed vertically, horizontally or obliquely, however, the top 20 metres of the water column (where most eulachon larvae are captured) are most intensively sampled by horizontal or oblique tows as described above.

SURVEY TIMING AND DESIGN SPECIFICS

OCEAN SURVEYS

It is important to time ocean ichthyoplankton surveys at least 4 to 6 weeks after completion of major eulachon spawning activity. If surveys are timed too early, significant numbers of eulachon larvae may still be present in rivers. If plankton net samples are to be collected and compared annually, it is equally important to time surveys consistently. Larvae should be sampled at a similar stage of ocean dispersal or during a time period when most larvae are approximately the same size (6 to 10 mm). Estuarine circulation is prevalent in most eulachon-bearing inlets where the ocean densities of eulachon larvae are usually highest off the mouths of eulachon-bearing rivers and then decrease in a seaward direction until reaching an inlet sill or junction (Fig. 4). There is evidence of some degree of larval retention inside BC's inlets from mid-April until August (McCarter and Hay 1999).

We have conducted several exploratory larval eulachon surveys along the central coast of BC using a 57 cm diameter bongo net. A series of oblique, plankton net tows, filtering seawater to a 20 m maximum depth, were routinely conducted over a 10 to 12 day period. These surveys were conducted between mid-April and June of 1994, 1996 and 1997 and covered large geographic areas. Eulachon larval distributions, ocean densities and dispersal patterns were estimated by identifying and counting larval fish species and measuring the lengths of eulachon larvae captured at each sampling station (McCarter and Hay 1999). An adequate number and distribution of ichthyoplankton sampling stations is necessary to delineate larval eulachon dispersal patterns (i.e. when larvae are emerging from several adjacent eulachon-bearing rivers). An intensive larval survey confined to the immediate estuarine areas of a single, eulachon-bearing river is also another survey design option. A much smaller number of sampling stations can be visited at regular intervals (i.e. daily) over the period of larval outdrift. In addition to broad area, synoptic surveys, we have also conducted several series of discrete depth, tows in selected inlets (0 to 35 m vertical depth at 5 m intervals) adjacent to eulachon-bearing rivers to examine larval depth distribution over a 24 hour period. The results of these experiments and comparative net efficiency trials are discussed in a subsequent section.

RIVER SURVEYS

On the Fraser River, we have routinely conducted oblique tows and a series of discrete depth, tows at the surface (0 m), 5 m and 10 m sampling depths using a small, 19 cm diameter bongo net described above. Sometimes only oblique tows were conducted when survey time was limited (sampling the entire surface waters to a maximum depth of 10 m). Bongo nets towed at discrete depths were lowered quickly at a rate of approximately 1 m/sec, maintained at a fixed depth for approximately 6 minutes and then raised at the same rate. The purpose of these tows was to examine vertical distribution patterns as drifting larval eulachon can be patchy at peak times, with some larvae concentrating at specific depths or positions on some sites in the river (see Figs. 5 and 6). Oblique tows, on the other hand, provide a consistent sampling time interval at each sampling depth by lowering the net to a 10 m depth during the first 30 seconds and then slowly raising the net at a constant rate over the remaining portion of a 6 minute tow.

Sampling sites on the lower Fraser River were established in mid-river positions with additional tows conducted near the north and south banks of the river. The deeper, swifter sides of the Fraser River tend to produce higher larval eulachon densities than either mid-river or shallower, slower moving sides of the river despite higher sediment loads. The general geography and hydrology of the river should be considered when selecting stations. On the lower Fraser River, for example, most tributaries enter the mainstem on the north side and some of these rivers drain major glaciers or snowpacks. In BC, there is a connection between spawning eulachon and certain rivers that have strong, spring freshets and drain major glaciers or snowpacks (Hay and McCarter 2000). Sampling stations should be strategically distributed in rivers as there is convincing evidence that spawning distributions can also vary between years. Cross-river dispersal or horizontal mixing is gradual in the lower 50 km of the Fraser as the lower portion of the river is relatively channelized (for navigation) with minimum meander. It is therefore important to establish several sampling sites along cross-river transects. Commercial gillnet vessels, survey launches and whalers have been deployed on the Fraser River to tow small bongo nets in a bi-weekly sampling design covering 5 major sampling areas over a 7 week period from late April until mid-June. A small, over-the-side boom and towing block can be rigged on most gillnet vessels and the nets can be hauled with a 3/16 inch stainless steel wire cable and

hydraulic gillnet drum. Government survey launches have utilized a small, midship davit and scientific winch to tow the plankton nets with a 10 to 15 kg cannon ball weight. The contents of both left and right cod-end buckets from each plankton net tow have been combined and preserved in 1 litre sampling jars. Eulachon assessments based on egg and larval surveys have been conducted annually on the Fraser River since 1995 (Hay *et al.* 1997, 2002).

DEPTH DISTRIBUTION OF LARVAE AND CAPTURE AVOIDANCE

OCEAN SURVEYS

We have conducted several series of discrete depth, plankton net tows at 10 m depth intervals between the surface and 100 m depth from 1985 to 1997 in several BC open water and estuarine locations. A small, (0.3036 m^2 opening) mid-water, Tucker trawl (0.35 mm mesh) with a 3 net entrance, opening and closing trip messenger, mechanism has been deployed. Multiple bongo nets, rapidly lowered and raised on the same cable (0.2544 m^2 and 0.0283 m^2 openings) have also been used in the experiments. Most eulachon larvae have been captured in the surface waters between 0 and 15 m depth. Considerably fewer larvae were caught at depths of 15 to 35 m and almost none below 35 metres. Multiple bongo nets towed simultaneously on the same cable, captured fish larvae directly proportional to the areas of each of the net openings. In general, we found that the ocean densities of eulachon larvae were greater near the surface waters during night plankton tows than during daytime tows. Continuous advection of pulses or waves of larvae through the sampling areas, however, can often obscure vertical or diurnal distribution patterns. Deflection of larvae near the stern wash of a vessel and capture avoidance by large, developed larvae in the undisturbed surface waters off the side of a vessel during daylight may account for some of the consistently lower ocean density measurements. The sizes of larval eulachon were significantly smaller in daytime catches than night catches. Larvae captured in surface waters were also consistently smaller than those captured at deeper depths. Capture avoidance of large, developed larvae is a significant factor considering eulachon larvae greater than 30 mm in length are rarely captured in bongo net gear (McCarter *et al.* 1986). Ichthyoplankton surveys should therefore be conducted early in the season when larvae are relatively small (< 15 mm) and sampled with oblique tows (covering 0 to 20 m sampling depths) so that deflection and capture avoidance by large larvae in the surface waters is minimized. This may be important when comparing larval density measurements between years as this potentially overlooked sampling bias can have a distorting effect if surveys are timed inconsistently or if sampling is confined only to near surface waters (0 to 3 metres depth).

The nature of seawater filtered through a plankton net during an ichthyoplankton survey can sometimes effect a net's efficiency or ability to catch fish larvae. Seawater turbidity such as the milky-coloured surface waters adjacent to glacier-fed rivers can be highly variable in some survey areas. Sharp borders between turbid and clear water can be observed in many BC inlets. The measured densities of larval eulachon in these waters often decline at these locations and also where inlets merge with other channels or passages. Plankton blooms of small diatoms (alive and/or dead) can also occur in survey areas. Planktonic, filamentous material can quickly obstruct a plankton net's filtering apertures, creating a pressure wave in front of a net and dramatically decreasing a net's effectiveness. The problem is often detected by the persistence of unusually low flow meter readings measured at the entrance to the net. Ocean densities of larvae

can be grossly underestimated under these circumstances as water and larvae are deflected away from net openings. In addition, considerable net washing is required after each tow in order to clear obstructed net meshes. It is therefore best to avoid these circumstances by careful survey design and timing to avoid spring plankton blooms.

RIVER SURVEYS

Capture avoidance in rivers is not a major problem as the small, newly hatched eulachon larvae and embryonic eggs are carried quickly and passively down river. Plankton nets can be towed against the current while the vessel's position is held steady with the river bank. Obstruction of net meshes and deflecting pressure waves rarely develop in front of our bongo nets over the period of a 6 minute tow. General Oceanic flow meter readings of each plankton net tow are relatively constant and usually vary more by small, variations in tow durations. Significant sand, silt and small woody fibrous debris, however, can be collected in a net's cod-end buckets especially when rivers are approaching flood stages and carrying heavy sediment loads. Our river plankton nets, nevertheless, are easily washed down as the net meshes do not become obstructed with filamentous or fibrous material to the same extent or frequency as can occur during ocean surveys. Egg and larval densities in rivers can consequently be determined at a variety of sites, depths and times. These density measurements can be combined appropriately with daily river discharge data (Inland Waters, Water Survey of Canada) to produce egg and larval production estimates (Hay and McCarter 2002). Typical counts of eulachon larvae have generally ranged from 0 to 3,000 larvae per 6 minute tow. Ninety-nine percent of Fraser River samples collected between the years 1995 and 2002 have produced counts between 0 and 500 larvae per 6 minute tow.

It is sometimes anticipated that plankton net surveys in rivers can determine the exact locations of eulachon spawning sites. Exact river spawning locations, however, are difficult to determine by plankton net surveys alone. The cumulative down river effect of several separate spawning events within the same or different time periods can often obscure distribution patterns even with extensive spatial and temporal sampling coverage. The densities of eulachon larvae are generally higher at down river stations than up river stations, however, up river sites can sometimes produce higher density estimates immediately after hatching and are often characterized by the capture of large numbers of embryonic eggs as well as very small, curled larvae. Another aspect of sampling in the lower portions of large river estuaries is related to the presence of a dynamic system of tidal influxes and surface currents. Tidal penetration upriver can pose a potential problem of cumulative sampling coverage as the deeper, more saline waters may penetrate and retain or delay eulachon larvae in some lower estuarine sampling sites. We have tried to avoid sampling under these circumstances (during maximum flood tides), but occasionally this is unavoidable.

SAMPLE COLLECTION AND PRESERVATION

The recovered nets from each plankton net tow should be thoroughly washed down with water and the contents of cod-end buckets preserved in a dilute, 1 to 2% formaldehyde solution (5% commercial formalin). Sampling jars can be filled and capped on shore, ahead of time with

small volumes (10 to 50 ml depending on the size of the jar) of commercial strength (37%) formaldehyde to avoid spillage onboard vessels. Jars can then be topped up with water after the plankton net samples have been collected. A relatively low formalin concentration is required to fix individual fish eggs and larvae and this field practise has also helped to reduce a technician's exposure to any lingering formaldehyde odours and fumes in the laboratory. A deck hose is preferable for washing large plankton nets and a wash-down bucket or a two litre, plastic juice pitcher works well for smaller nets. A sufficient degree of water pressure or force is required to clear obstructed meshes. Simply dipping the plankton nets up and down in the water is often not sufficient to clear obstructed meshes. The cod-end buckets should be easily uncoupled and can be constructed from standard ABS (Acrylnitrile butadiene styrene) or PVC (Polyvinyl chloride) pipe with threaded plumbing fittings and clamped to the nets with stainless steel hose clamps. We have used standard 2 inch inside diameter (ID) cod-end buckets for the smaller, 19 cm diameter bongos and 3 inch ID cod-end buckets for the larger, 57 cm diameter bongo nets. Drainage holes, two cm in diameter, should be drilled in the cod-end buckets and Nitex® mesh screens can be applied with ABS or PVC cement to cover the holes. An alternate method is to heat weld a stainless steel sieve to cover the holes, but this method is time-consuming and expensive. Cod-end buckets can be quickly and inexpensively constructed with materials obtainable at a building or plumbing supply store. A ready supply of spare, cod-end buckets and plankton nets is advisable when conducting ichthyoplankton surveys in remote areas. The location, date, time and depth (m) of each sampling site and the flow meter readings of each plankton tow should be recorded on jar labels and in water-proof paper field notebooks.

LABORATORY PROCESSING OF ICHTHYOPLANKTON SAMPLES

Plankton samples are transferred from 1.0 litre field jars to 150x20 mm glass petri dishes where counts of eulachon embryonic eggs, larvae and other larval fish species can be conducted. Plankton samples collected in rivers often contain considerable amounts of sand, silt and woody debris that must be washed and sieved through 2 mm and 600 micron sieves to reduce the volume of debris and silt to be searched (Fig. 7). The contents of the upper 2 mm sieve are discarded and the contents of the lower 600 micron sieve are washed into a 150x20 mm petri dish with a small water hose, wash bottle and small spatula. A grid marked on the bottom of the petri dish facilitates systematic inspection of each grid quadrat. Care must be taken not to transfer a large portion of high-debris samples at one time such that embryonic eggs and larvae can become hidden by debris. A small spatula and a water-filled wash bottle can be used to disperse clumps and dilute the sample density in the petri dish. Direct counts of fish larvae are conducted with fine, "insect handling" forceps under a dissecting microscope adjusted to low magnification. A transfer, micro-pipette can be useful for counting eulachon eggs. A dissecting microscope is required to inspect river samples because eulachon embryonic eggs (0.8 to 1.0 mm in diameter) and newly hatched larvae (4 to 6.5 mm in length) are often difficult to see with the unaided eye. A magnifier-illuminator can be used for ocean plankton samples where eulachon larvae are often larger (5.5 to 25 mm in length) and can easily be spotted and distinguished from other plankters. Raw plankton samples collected from mid-April to June in most **inside** BC inlets and steep-sided fjords at 0 to 20 m sampling depths are relatively free of other plankters such as copepods and euphausiids. Other BC plankton sampling sites, however, with high

degrees of exposure to the open ocean can frequently produce a rich variety and quantity of plankters in the nets, significantly increasing laboratory, processing time.

Standard sub-sampling with a Folsam plankton splitter or a beaker technique (Van Guelpen et. al. 1982) and estimation of larval numbers is required for any sample that exceeds approximately 1000 larvae. It is best to avoid excessive counts of 1000 or more larvae. Survey design adjustments such as the seasonal timing of the survey, the duration of the plankton net tows, or the size of the plankton net openings can facilitate in lowering excessive counts. We have standardized on a 6 minute tow with a total net opening of 0.0566 m^2 (left and right sides combined) using a 19 cm diameter bongo that we routinely deploy on the Fraser River. A larger net opening (0.2544 m^2) afforded on a 57 cm diameter bongo frame and a 6 minute tow duration is effective for ocean surveys where eulachon larvae are much more dispersed. Water filtering volumes during each tow should remain relatively constant throughout a survey to insure a consistent minimum measuring resolution of larval densities discussed earlier. Excessive counts of 1000 or more larvae can be avoided by filtering smaller volumes of water while still permitting a sufficient sampling volume for the detection and measurement of low larval densities. Excessive sub-sampling of samples in the laboratory is not an effective use of time, as much of the effort collecting samples in the field can be compromised by the error introduced with excessive sub-sampling in the laboratory. We have never sub-sampled more than 1% of the total number of samples collected from any survey and we rarely conduct direct counts of over 1000 larvae from any single sample.

In the past we have used a Folsam plankton splitter to sub-sample a small number of ocean samples when herring larvae have been collected in large quantities at herring spawning sites within a day or so of hatching (ie >10,000 larvae per 6 minute tow near Denman Island in 1991). Most herring ichthyoplankton surveys have been timed at least a week or so after hatching and consequently have avoided the need for laboratory estimation of excessively high numbers of captured larval fish. Most larval eulachon counts obtained from ocean samples collected between 1985 and 1997 have not been sufficiently high to warrant sub-sampling either (99 percent of samples have had less than 500 eulachon larvae in each 6 minute tow). Direct counting of eulachon larvae was therefore chosen for **all** ocean samples collected, not only because larval numbers were relatively low, but also because samples usually were relatively free of large quantities of other plankters and debris. A beaker sub-sampling technique, however, has been used on a number of larval eulachon samples collected from the Fraser River because of problems of clumping with sand, woody and other fibrous material that still remains after sieving. The procedure involves constantly stirring a sample while pouring equal amounts into two beakers repeatedly, back and forth several times until the sample is divided equally into two fractions. Although stratification can develop quickly, layers tend to be split equally without bias. We have found that samples collected at 10 m depth on the Fraser River have less fibrous material than those collected at the surface or 5 m depths and that these samples are more suitable for splitting using this technique. A typical count from a single fraction or "plankton split" would be in the range of a few hundred larvae rather than a few thousand larvae, if directly counted. Final estimations are determined by the product of the sub-sample count and the number of splitting fractions (usually only two or four).

Larval identifications can be determined by descriptions and drawings in Garrison and Miller (1982) or Matarese *et al.* (1989). Commonly abundant fish larvae can be sorted into

categories corresponding approximately to taxonomic families: Osmeridae (mostly Eulachon, *Thaleichthys pacificus*), Clupeidae (mostly Pacific herring, *Clupea pallasi*), Gadidae (undistinguished codfishes), Stichaeidae & Pholidae (undistinguished pricklebacks & gunnels), Ammodytidae (Pacific Sand Lance, *Ammodytes hexapterus*), Hexagrammidae (undistinguished greenlings & Lingcod), Cottidae, Cyclopteridae & Scorpaenidae (undistinguished sculpins, snailfishes & rockfishes), Agonidae (undistinguished poachers), Pleuronectidae (undistinguished flatfishes), Bathylagidae (deepsea smelt, mostly Northern Smoothtongue, *Leuroglossus stilbius schmidti*). More accurate identification to the species level is time-consuming (requiring myomere and fin ray counts) and is not routinely done for every fish larva counted. The most important skill is to be able to distinguish small eulachon larvae (4 to 30 mm in size) from a variety of similar appearing and abundant marine larval fish species, particularly herring, sand lance and deepsea smelt. Plankton samples collected in eulachon-bearing rivers contain very few other larval fish species and most of these are very different in appearance (e.g. sculpins, starry flounders, salmonid fry and lamprey). Occasionally, soft, deteriorating eulachon larvae can be observed in some samples and sometimes only head portions are counted. It is presumed that some of these larvae were dead at the time of capture and were subsequently damaged during plankton net filtering or laboratory sieving. Most eulachon larvae captured, however, were in good condition and appeared to have been alive and healthy at the time of capture. A few dead eggs (often shrunken and white or pale green in color) can sometimes be found in river samples and should not be confused with other spherical organisms such as green algae. Most fish eggs captured in the plankton nets, contained fish embryos at very similar developmental stages close to hatching. It is likely that these embryonic eggs were alive and incubating at the time of capture. We therefore have combined all counted fish eggs and larvae when determining total production estimates (Hay *et al.* 2002).

After counting is completed, eggs and larvae can be placed in sample vials and preserved for subsequent measuring, drying and weighing. Length measurements of individual larvae can be efficiently obtained with the use of a dissecting microscope fitted with an ocular micrometer. Larval fish species identification can also be confirmed at this time. Corresponding larval dry weights to the nearest 0.001 mg can be acquired by drying the measured larvae on small weighing pans over night, in a 100 °C oven and individually weighing the dried larvae on an electronic balance the following morning. The total plankton contents of ocean samples can easily be suctioned with a Buchner funnel, dried in a 100 °C oven for 18 to 20 hrs and weighed to the nearest 0.01 g on an electronic balance to obtain a total dry plankton weight of each sample.

EGG AND LARVAL DENSITY MEASUREMENTS AND ADULT ABUNDANCE ESTIMATIONS

OCEAN SURVEYS

Larval eulachon density (**D**) measurements (number/m^3) can be determined for each sampling station by calculating the quotient of the laboratory count (**N**) and the corresponding volume (**V**) of seawater (m^3) filtered through the plankton net:

$$\mathbf{D = N / V} \qquad \mathbf{(1)}$$

The volume of water filtered through the net is determined using a General Oceanics® equation:

$$\mathbf{V = (A \cdot F \cdot K) / 999{,}999} \qquad \mathbf{(2)}$$

where:

V = volume of water filtered through the plankton net
A = area of net opening (m^2)
F = number of revolutions recorded by a General Oceanics® model 2030R flow meter
K = rotor constant

and:

high speed rotor constant = 26,873 (7.0 cm rotor)
low speed rotor constant = 51,020 (16.5 cm rotor)
large, 57 cm bongo net opening = 0.2544 m^2 or 0.5088 m^2 for both nets
small, 19 cm bongo net opening = 0.0283 m^2 or 0.0566 m^2 for both nets
Scor, 60 cm net opening = 0.2827 m^2

The total ocean surface area (m^2) of each region surveyed can be estimated using a raster or vector based Geographical Information System (GIS), surface area calculating software or hydrographic chart and planimeter. A simple area expansion method can be used to approximate the total number of eulachon larvae that may have been present in a region at the time of the survey. The ocean surface area (m^2) represented by each sampling station can be determined by dividing the total estimated regional area (m^2) by the total number of equally distributed sampling stations. An estimate of the total numbers of fish larvae can be determined for each region by the sum of the product of each station's represented area (m^2), the oblique sampling tow depth (m) and the corresponding larval density measurement (number/m^3). Larval density maps (Figs 8 to 11) of each region can be constructed using plotting software. The co-ordinates of each sampling station can be electronically plotted on digitized hydrographic charts with plotting circles proportional to measured larval fish densities. Reliable measures of total spawner abundance, however, cannot be obtained from ocean ichthyoplankton surveys because of sampling deficiencies (amount of effort required to cover large areas in short periods of time), capture avoidance by large (growing) fish larvae and difficulties incorporating daily larval fish mortality rates. Larval fish surveys, nevertheless, can provide indications of abundance as long as survey timing and sampling protocols are consistent from year to year. At the very least, ocean ichthyoplankton surveys can provide evidence of "presence or absence".

RIVER SURVEYS

Abundance estimations of spawning eulachon runs based on egg and larval surveys conducted in rivers rely on several key assumptions: (1) Passive, unidirectional advection of embryonic eggs and larvae downstream, (2) Negligible plankton net avoidance by deflection, (3) Sufficient and representative spatial sampling downstream of spawning, (4) Sufficient and representative temporal sampling over the period of hatching and downstream drift, (5) Reasonably accurate eulachon egg and larval density estimations (number/m^3), (6) Reasonably

accurate daily river discharge measurements (m^3/s) and (7) Reasonably accurate relative fecundity and sex ratio estimates of spawning eulachon in rivers.

The basic concept of estimating the number of spawning fish from a measure of a stock's annual egg and larval production is simple. We have used a common equation that is the basis for estimating the spawning stock size of many fisheries, including the "escapement" method used for Pacific herring (e.g. Schweigert *et al.* 1998). The general equation has been modified to estimate the approximate size of an eulachon spawning run in a river as follows:

$$B = P / (R \cdot S) \qquad (3)$$

where:

B = biomass of the spawning stock.
P = total egg production of the stock.
R = relative fecundity or number of eggs per unit weight of spawning females.
S = sex ratio or proportion of females in the spawning stock.

Relative fecundity (**R**) and sex ratio (**S**) are determined in the laboratory and are discussed in detail in **section (3)**. Production (**P**) is estimated for each time period (**t**) as the product of the mean density (**D**) of eulachon eggs plus larvae (numbers/m^3), the river discharge (**V**) (m^3/s) and the interval (**I**) or duration of the time period (**t**) in seconds:

$$P_t = D_t V_t I_t \qquad (4)$$

Bi-weekly, multiple depth (and variable position), ichthyoplankton sampling in the Fraser River has provided a pool of 30 to 45 sampling measurements per week for the determination of mean densities in each area. We have applied a bootstrapping procedure described in section (1) to estimate mean egg and larval densities in the Fraser River with 95% confidence intervals.

(1) Bootstrapped egg and larvae density estimations

Eulachon embryonic egg and larval densities in rivers are measured in a similar manner to ocean surveys. Laboratory counts of embryonic eggs and larvae from each sample are converted to density measurements by determining the volume of water filtered during each respective tow (General Oceanics® flow determination equation 1 above). Since measured density, sampling distributions are typically skewed, data are log transformed and a bootstrapping procedure is used to determine the error structure around the estimates (Hay and McCarter 2002). For each bootstrap sample we let n=1000 which exceeds the suggested minimum of 200 suggested by Efron (1993). Bootstrap estimates (sampling with replacement) were generated for each sampling period (i.e. for each of 7, one week periods encompassing the total Fraser River egg and larval outdrift period). The means and standard deviations were estimated from the 1000 bootstrap replications for each week. Confidence limits were estimated for weekly, as well as for cumulative sampling periods.

(2) River discharge measurements

Fraser River daily, discharges (m^3/sec) were obtained from Inland Waters, Water Survey of Canada. The gauging station is located at Hope, BC. The section of the river we have found to be most representative of overall eulachon egg and larval densities is the South Arm of the

Fraser River and includes the Deas Island and Tilbury Island sampling stations. There are a number of sources of fresh water contribution between the Hope gauging station and the South Arm egg and larval sampling areas. Thompson (1981) estimates that the discharge in the vicinity of the study area is about 30% greater than the discharge estimated at Hope. A 25% diversion of river water (Thompson 1981) through the North Arm of the Fraser River must also be included in the calculations. Since river diversion approximately equalled contribution, it was decided that daily flow measurements from the gauging station at Hope (during the 7 week egg and larval sampling period) would be applied to South Arm samples without any correction. Samples collected in the North Arm of the Fraser River, however, were corrected by a 25% flow diversion rate.

(3) Relative fecundity estimations

The relative fecundity of spawning eulachon can be determined gravimetrically in the laboratory. Eulachon can be sampled either in a fresh state or in a previously frozen, thawed state. Each fish is blotted dry and the total body weight is determined to the nearest 0.1 gram using an electronic balance. The standard length of each fish is recorded to the nearest millimetre. Whole ovaries are extracted from ripe or nearly ripe females. Females should not be in a running ripe condition as some eggs may have already been extruded due to fishing practises and fish handling and storage procedures. Extracted ovaries should be preserved in a 3.7% formaldehyde solution for at least 2 weeks to harden the eggs. Separate preservation jars should be used for each pair of ovaries. After the hardening period, the ovaries should be rinsed in seawater and dissected with fine "insect handling" forceps under a dissecting microscope. Three sub-samples of 100 eggs can be extracted from 3 different locations in the ovary. The three sub-samples and the pair of ovaries from which they were extracted should be vacuumed-dried for one minute in a Buchner funnel on a damp circle of fine-mesh (0.05 to 0.10 mm) Nitex® material or filter paper and then separately weighed on an electronic balance. The whole, preserved ovaries can be weighed to the nearest 0.01 gram while the 100-egg sub-samples should be weighed to the nearest 0.1 mg on a precision electro-balance (e.g. Cahn®). It is important to immediately weigh the 100-egg sub-samples and the whole ovaries at the same time before any further evaporation may cause weighing instabilities (similar relative moisture content). If any of the egg weight sub-samples differ from each other by more than 10%, the sub-sample of eggs should be returned to the ovary and the process repeated. The mean weight of a preserved individual egg is estimated by the sum of the three egg weight sub-samples divided by 300. The fecundity (number of eggs per individual female) is estimated as the preserved, total ovary weight divided by the mean preserved, individual egg weight. The relative fecundity (number of eggs per gram of body weight) is calculated as the fecundity estimate divided by the total body weight. The sex ratio is determined by the total count of males and females in a random sample. We have estimated the relative fecundity of 624 female eulachon collected from several eulachon-bearing rivers between the years 1993 and 1998. Most 100-egg sub-samples collected from these fish have weighed between 30 and 70 mg indicating various degrees of egg hydration or degrees of female ripeness at the time of sampling. The mean relative fecundity of these fish has been estimated at between 350 and 400 eggs per gram of eulachon.

DISCUSSION

LARVAL SURVEYS AS INDICATORS OF SPAWNING ORIGINS

One of the primary objectives of ocean larval surveys has been to use the larval distribution patterns to corroborate the existence of spawning runs in different rivers along the BC coast. For most rivers that are known to have spawning runs, we have found eulachon larvae in the adjacent marine and estuarine waters. In some instances, we have found additional concentrations of larvae that appeared to originate from small rivers that were previously not known as eulachon-bearing rivers (see Table 1). We confirm that some rivers are indeed used for eulachon spawning and suggest that several more, not previously known to be eulachon spawning areas, are also used for spawning as they are apparent sources of eulachon larvae.

The results of six larval herring surveys (Hay and McCarter 1997) and three larval eulachon surveys (McCarter and Hay 1999) between 1985 and 1997 have confirmed the presence of nearby spawning eulachon runs. Broad, synoptic larval surveys conducted among the highly mountainous coastal inlets of BC have repeatedly produced several distinct eulachon larval distribution patterns. Some areas around the Strait of Georgia, off the west coast of Vancouver Island and near the Queen Charlotte Islands have also been examined during ichthyoplankton surveys directed at describing the distribution of Pacific herring larvae. These other larval surveys have been conducted in April and May and found virtually no eulachon larvae in these outer regions that were further away from the Coast Mountain Range. These observations reinforce the conclusion that eulachon spawning is mainly confined to coastal rivers that have a distinct spring freshet and drain major glaciers or snowpacks (Hay *et al.* 1997).

BIOLOGICAL STATUS OF DIFFERENT EULACHON RUNS

The biological uniqueness of different eulachon populations or runs remains uncertain. Recent genetic evidence, based on mitochrondrial and micro-satellite DNA analyses (McLean 1999, McLean *et al.* 1999) indicates that there are few differences between any rivers in BC and virtually none between geographically adjacent rivers. These results agree with other approaches that examined and compared the elemental analyses of otoliths from different populations (Carolsfeld and Hay 1998). The general (but still preliminary) conclusions from the genetic and otolith chemistry analyses are that there are few, if any, differences among eulachon populations. In contrast, there are a number of biological factors, which appear to indicate that there are striking differences among different populations. The most apparent is simply the geographical discontinuity of different spawning runs, different spawning times, and the apparent 'homing' of each run to individual rivers. It is well established that there are biological differences among many different salmon runs (Hasler 1966), so it is difficult to rule out the potential for similar types of variation among eulachon runs. Perhaps the most striking apparent difference among different eulachon populations is the timing of spawning. In some rivers, such as the Kitimat or Kemano, the time of spawning is relatively early, beginning in early March and in others, such as the Fraser or Klinaklini, the timing is later, beginning in April or May. Based on concepts developed from observation of spawning of Pacific salmon, the timing of spawning runs should be biologically adapted to each river. If so, and if the same model is applied to eulachon, then each population would be adapted to each river. Therefore, until we better understand the

biological and genetic variability (or lack of it) among different eulachon populations, we are not prepared to ignore any population differences among different rivers or estuaries.

IMPLICATIONS OF LARVAL SURVEY RESULTS FOR EULACHON STOCKS

The distributions of larval eulachon described in McCarter and Hay (1999) confirms that the number of spawning areas used by eulachon is limited. In some of those instances, however, where several rivers or streams drain into the same inlet (e.g. Klinaklini and Franklin rivers flow into Knight Inlet, or the Kitlope, Kemano and Kowesas rivers flow into Gardner Canal) we cannot be certain about the relative contribution of specific rivers to the numbers of larvae we observe in the adjacent estuarine or marine waters. Indeed the close proximity of different potential spawning rivers casts doubt on the capability for adjacent rivers to maintain distinct biological stocks. For instance, following the basic salmon life-history model, it is not unreasonable to assume, a priori, that eulachon may home to individual rivers. Homing, however, must be preceded by imprinting at an earlier life history stage. Salmonid imprinting may occur at several stages, and the first stage is thought to involve some form of olfactory recognition of chemical constituents in the water just after hatching. Imprinting is not thought to occur during the egg stage, presumably because of the relative impermeability of the egg capsule. Therefore, if these constraints applied to eulachon, there would be no imprinting during the 2 to 4 week egg incubation stage. If eulachon imprinted after hatching, they probably would have to accomplish this rapidly, because in most instances larvae are advected rapidly to estuarine or marine waters. Given the flow rates in some eulachon-bearing rivers, the freshwater residency of newly hatched eulachon larvae would be measured in minutes or, at most, hours. This would provide very little time for larvae to imprint, compared to the much longer period of salmonid freshwater residency (measured in months or years). Furthermore, eulachon larvae constitute only a few milligrams of wet weight, whereas salmonid alevins, fry and smolts are thousands of times larger, and presumably have more biological capability (tissue and sensory organs) for imprinting. Therefore, we suggest that it is unlikely that eulachon imprint during their freshwater egg and larval stages. On the other hand, our larval distribution data indicates that larvae reside in estuaries for considerable periods, weeks and perhaps months, and may be retained there by estuarine circulation. This resident time could provide an opportunity to imprint, but if so, the imprinting would be to estuarine waters and not necessarily to the water discharged from specific rivers. Therefore we suggest that estuaries may be an important criterion for population configuration and that the numbers of different spawning runs could be determined (or limited) by the numbers of different estuaries. It also follows that annual variation in river discharges might lead to changes in the relative sizes of the eulachon spawning runs among rivers.

UTILITY OF LARVAL SURVEYS AS INDICATORS OF EULACHON SPAWNING RIVERS

Ichthyoplankton surveys are sensitive detectors of *small* spawning runs that might be missed by conventional fishing techniques (gill nets or seine nets) on adults. Substantial numbers of eulachon larvae can be caught in rivers and estuaries where no (or negligible) adult spawning is observed. Furthermore, the duration of the presence of larvae in adjacent estuaries seems to occur over a number of weeks, whereas the duration of spawning may be complete within days. A wide range of larval densities can also be measured using standard ichthyoplankton survey techniques, not only in rivers but also in estuaries, inlets and open ocean

areas, during an 18 to 20 week period (approximately April to August) 4 weeks after adult spawning has occurred. The basic technique is simple and requires a plankton net and a swept volume procedure.

UTILITY OF LARVAL SURVEYS AS INDICATORS OF EULACHON SPAWNING BIOMASS

Variation in vulnerability and catchability of adults can be a problem with other assessment techniques that use seines, trawls, gill nets or traps. Ichthyoplankton catchability, however, is relatively constant, as most targets are small (< 15 mm), oceanographically dispersed and unable to avoid the nets. Fishing 'skill' usually is not a complicating factor in capturing larvae so catchability or sampling variables are minimal. For these reasons, larval samples may be better 'unbiased' estimates of the population than samples from other gear types. Variations of standard ichthyoplankton surveys are currently used to assess the abundance of Fraser River adult eulachon spawning biomass (Hay *et al.* 1997, 2002). These surveys have utilized replicate sampling and bootstrapping techniques to derive consistent estimates with relatively tight confidence intervals.

Ocean surveys (McCarter and Hay 1999) on the other hand, were conducted primarily to assess larval distributions, rather than biomass. The main limitation of ocean data is that we cannot accurately estimate egg and larval mortality between egg deposition and larval capture. For these reasons, the estimates of total larval numbers are not a reliable index of spawning biomass. Regardless, there are some conservation concerns about eulachon and we felt it could be informative to estimate total numbers and then provide an approximate estimate of the spawning biomass required to produce the estimated numbers of larvae. The conversion from larval numbers to spawning biomass uses estimates of relative fecundity of about 400 eggs per gram of spawning female or about 800 eggs/g from the spawning populations (males included). Using this conversion, the biomass required to produce the observed larval eulachon density patterns was estimated.

It is also certain that the estimates of spawning biomass from ocean surveys are severe underestimates, mainly because they assume complete survival between the time of egg deposition and egg survival. Such an assumption is unreasonable, and the total mortality during this period could easily remove most of the larvae (e.g. 90% or 99% or more). We are aware of only a few biomass estimates from rivers in the central coast of BC (McCarter and Hay 1999). An estimate was made for the Kitimat River in 1993 (Pedersen *et al.*, 1995) of about 23 tonnes (based on an estimate of the number of discharged larvae at 5.7×10^9 and a relative fecundity of 250 egg/g). From aerial surveys, Triton Consultants (1990 and 1991) estimated a mean spawning escapement of 4.96×10^6 fish plus 1.875×10^6 fish taken in the fishery. At an approximate mean weight of about 50g/fish, the total spawning run (before catch) would have been about 340 tonnes, and this estimate was regarded as conservative because it did not include fish that entered and left the river prior to the survey, or after the survey. In 1991 eulachon may have spawned in other rivers in the Gardner Canal, such as the Kitlope and Kowesas, and their spawning biomass is unknown. Therefore we can only guess at the total biomass but it seems probable that the upper Gardner Canal, into which drain 3 major eulachon rivers (Kemano, Kitlope and Kowesas) could support eulachon spawning populations of 500 to 1000 tonnes or more. If so, the 1997 estimate of spawning biomass from larval surveys of 113 tonnes (and

which includes the Kitimat and Kildala Rivers) would represent about 10 to 20% of the spawning biomass in 1991. By presenting these estimates we do not mean to imply that there was a decrease in biomass between 1991 and 1997, and we do not mean to suggest that any conclusions can be drawn about larval survival. Rather, we only suggest that the numbers of larvae that we estimated in the surveys is not unreasonable relative to the rough estimates of available spawning biomass.

LARVAL SURVEY INFORMATION AS CONTRIBUTIONS TO THE BIOLOGY OF EULACHON

We have observed that eulachon larvae mix and distributions overlap with other eulachon larvae originating from several eulachon spawning rivers. This has occurred at the head of Knight Inlet, Dean Channel and Gardner Canal. In the central coast, eulachon larvae disperse and mix with other plankters in coastal areas during an 18 to 20 week period (April to August) four weeks after adult spawning has occurred. Based on modal variation in length frequency data, larvae grow from approximately 3 to 4 mm in size to 30 to 35 mm in size during this time period (McCarter and Hay 1999). Larval eulachon samples collected at the heads of inlets, adjacent to known eulachon-bearing rivers consisted predominantly of small, newly hatched larvae. Mean eulachon larval size (mm) generally increased at each sampling station in a seaward direction away from eulachon spawning rivers (Fig. 12).

Oceanographic features measured during the ichthyoplankton surveys suggest that both dispersion and retention mechanisms can be operating. Clearly there is dispersal of larvae as they discharge from the relatively small spawning areas in rivers (probably from a spawning or egg deposition area of between 0.1 and 1.0 km^2 in most rivers) to an area between 10 and 1000 km^2 for most larval survey, delineated areas. On the other hand, larvae appear to be retained in inlets to some degree, and these eulachon larval distributions seems to suggest a much higher degree of fjord residency than distributions of herring larvae, which are frequently captured at the same time of year. Consistently higher larval densities (eulachon or herring), measured on the left sides of coastal inlets (looking seaward), also seems to suggest an accumulation or retention effect (possible Coriolis effect) while larval samples collected at other inlet locations have showed a continuous dispersion effect due to estuarine outflow and wind and tidal influences (Hay and McCarter 1997).

The larval rearing environment in BC's deep, cold and remote inlets seems to be dominated more by physical factors than biological factors. The inlets and deep, steep-sided fjords surveyed are known to be relatively low in overall productivity as compared to the rich, productive offshore banks and adjacent nearshore areas exposed to open ocean. Therefore it is likely that some protection from predators is afforded in these inlets while eulachon larvae absorb their yolk sacs and gradually acquire the characteristics necessary to survive in open ocean environments. Furthermore, the confinement of eulachon larvae to the upper layers of relatively low saline water (resulting from estuarine circulation) would eliminate most stenohaline predators (e.g. most marine fishes and invertebrate predators). As a consequence, small spawning runs of eulachon may be more sensitive to ocean climate changes particularly those that impact freshwater discharge than, for instance, large spawning runs of herring that deposit vast numbers of progeny usually near the centers of highly productive areas.

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Table 1. List of known and other probable eulachon spawning and larval dispersal areas in British Columbia. Most areas were examined during *VECTOR*, April 25 - May 5, 1994, *R. B. YOUNG*, May 27 - June 7, 1996 and *VECTOR*, April 14 - 25, 1997 ichthyoplankton surveys (McCarter and Hay 1999) and during annual Fraser River assessments (Hay *et al.* 2002). River estuaries are ordered geographically, from southern to northern BC.

Known Eulachon Spawning Areas	Inlet Head & Larval Dispersal Areas
Fraser River	Lower Strait of Georgia
Squamish River	Howe Sound→ Lower Strait of Georgia
Homathko River	Bute Inlet → Johnstone Strait
Klinaklini & Franklin Rivers	Knight Inlet → Johnstone Strait
Kingcome River	Kingcome Inlet → Queen Charlotte Strait
Chuckwalla/Kilbella & Wannock Rivers	Rivers Inlet → Queen Charlotte Strait
Taleomey & Noeick Rivers	South Bentinck Arm → Burke Channel
Bella Coola River	North Bentinck Arm → Burke Channel
Skowquiltz River	Dean Channel (west side)
Kimsquit River	Dean Channel (head)
Kitlope River	Gardner Canal → Verney Passage
Kowesas River	Chief Mathews Bay → Gardner Canal
Kemano/Wahoo River	Kemano Bay → Gardner Canal
Kildala River	Kildala Arm → Douglas Channel
Kitimat River	Kitimat Arm → Douglas Channel
Skeena River	Telegraph Passage
Nass River	Portland Inlet
Stewart River	Portland Inlet
Probable Eulachon Spawning Areas (Based on larval surveys - see Figs. 8 to 11 and larval length frequency measurements)	Inlet Head & Larval Dispersal Areas
Stafford/Apple Rivers	Loughborough Inlet → Johnstone Strait
Kakweiken River	Thompson Sound → Johnstone Strait
Nekite River	Smith Inlet → Queen Charlotte Strait
Clyak River	Moses Inlet → Rivers Inlet
Kwatna River	Kwatna Inlet → Burke Channel
Unnamed river at the head of Cascade Inlet	Cascade Inlet → Burke Channel
Kainet or Lard Creek	Kynoch Inlet → Mathieson Channel
Khutze River	Khutze Inlet → Princess Royal Channel
Aaltanhash River	Aaltanhash Inlet → Princess Royal Ch.

Fig. 1. Major eulachon spawning areas in British Columbia (Hay *et al.*, 1997).

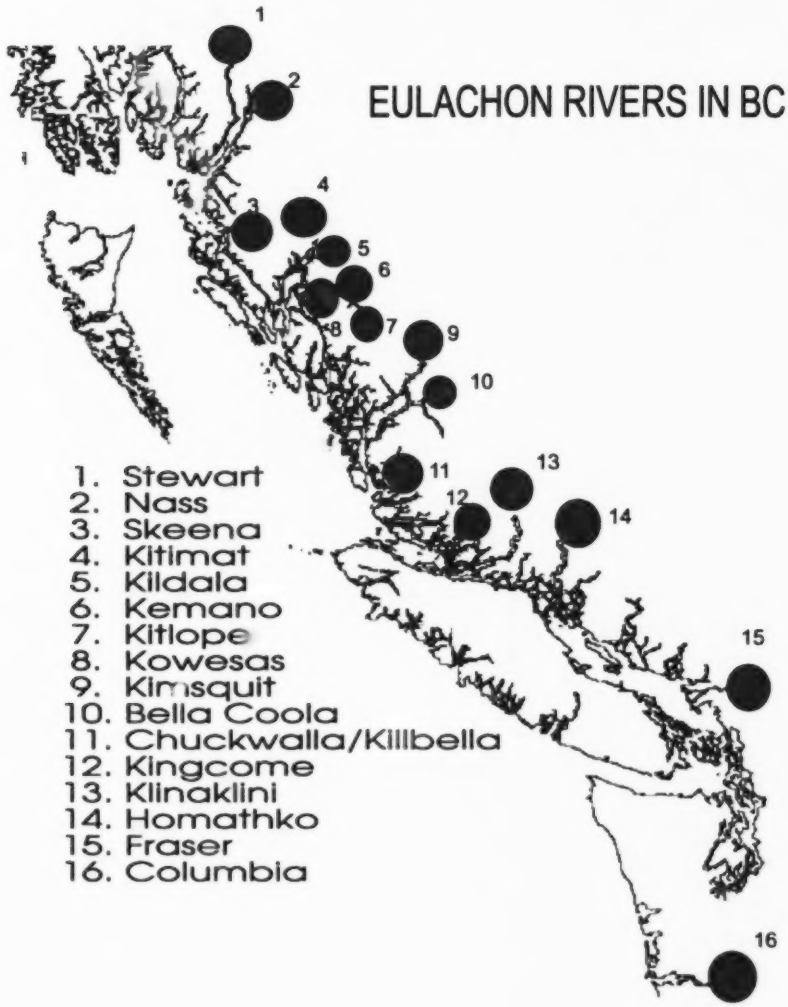
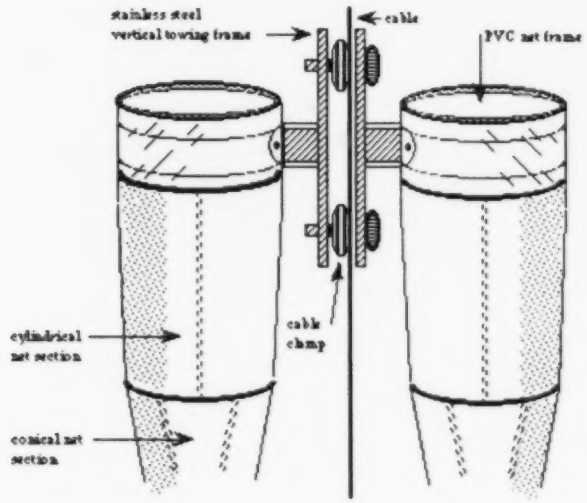


Fig 2. Schematic diagram of a typical bongo frame and paired plankton net design. 1. Shackled weight or V-Fin depressor. 2. Towing cable. 3. Aluminium or PVC bongo frame (57 cm or 19 cm inside diameter). 4. Stainless steel hose clamps. 5. Plankton net (0.35 to 0.6 mm aperture black Nitex® mesh) with dacron or nylon cloth collar. 6. Stainless steel hose clamp. 7. PVC or ABS cod-end plankton bucket. 8. Cod-end plankton bucket drainage holes.



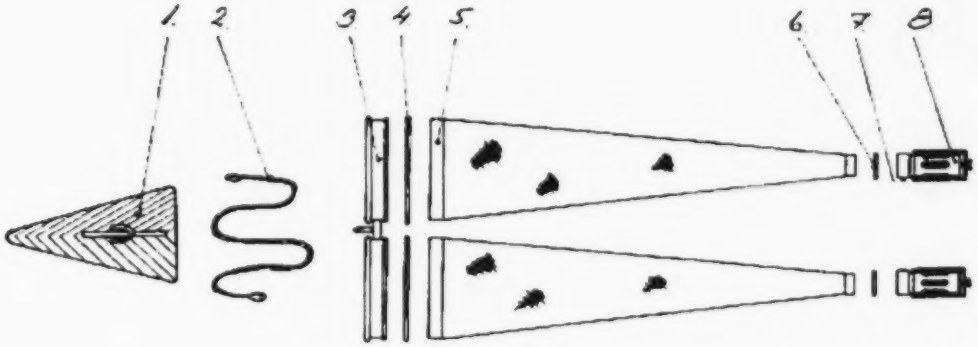


Fig. 3. Photographs of an oceanographic A-frame and block used to deploy bongo net gear. The paired plankton nets are washed down with a deck hose immediately after a flow meter reading is recorded.

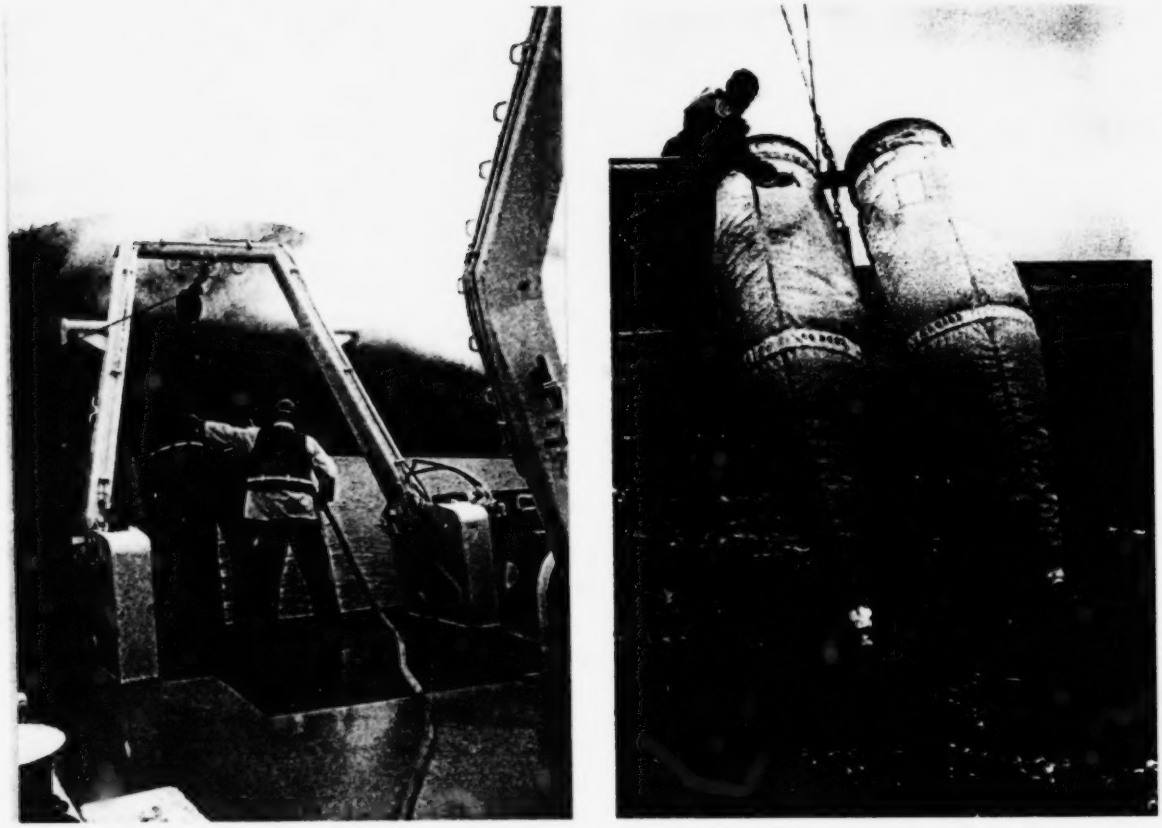


Fig. 4. Larval eulachon density map of the Johnstone Strait Region (JS) during April 25 – May 5, 1994 (Maximum density = 21.3 larvae/m3). A cross indicates a station where no eulachon larvae were captured. Densities decrease in a seaward direction away from eulachon-bearing rivers.

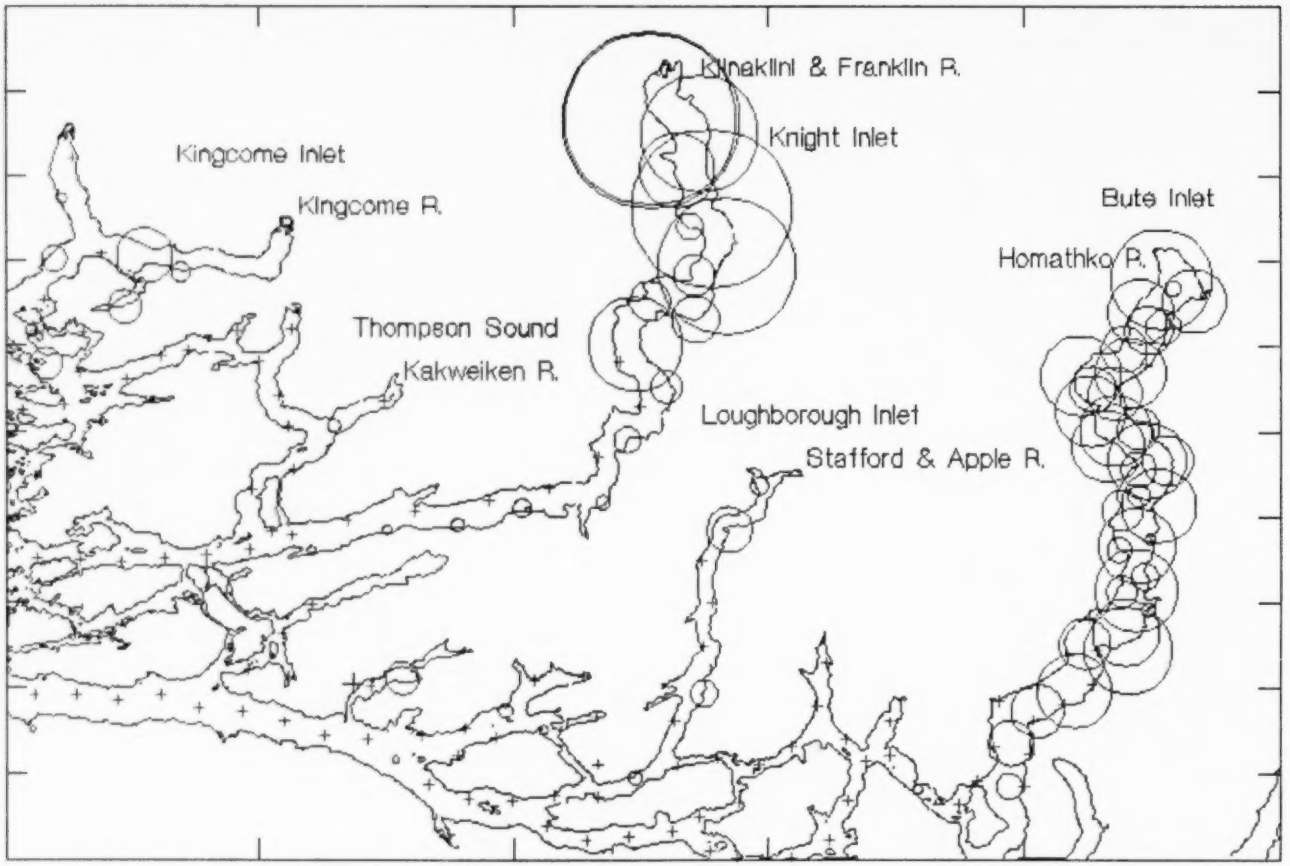


Fig 5. Larval eulachon density map of the lower Fraser River during the week of May 7-13, 1995 (Maximum density = 35.0 larvae/m3). An 'X' indicates a station where no eulachon larvae were captured. Density circles are shown by sampling depth (0, 5 and 10 metres) in the corresponding top panel plot.

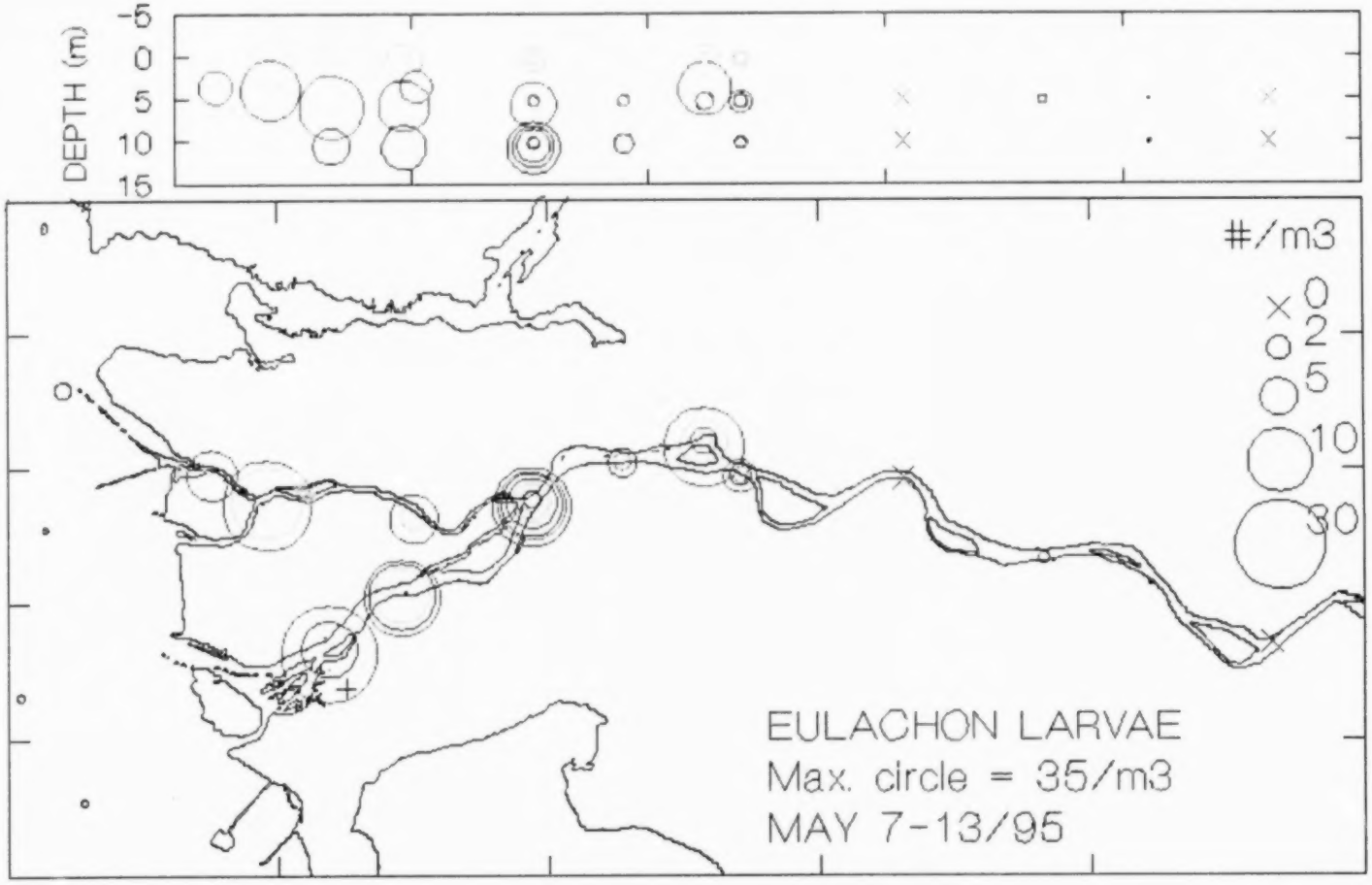


Fig. 6. An illustration and example of positional variation in egg and larval density between the north, middle and south sampling positions in the Fraser River. The x-axis shows the sampling positions (north, middle and south) and the y-axis shows the sample depth, from the surface to 10 meters. Each circle represents the results of a single tow with the radius of each circle proportional to the egg and larval density (n/m^3). While there is no apparent differences in abundance by depth, it is clear that the north and middle positions in the river had many instances of higher density than the south positions.

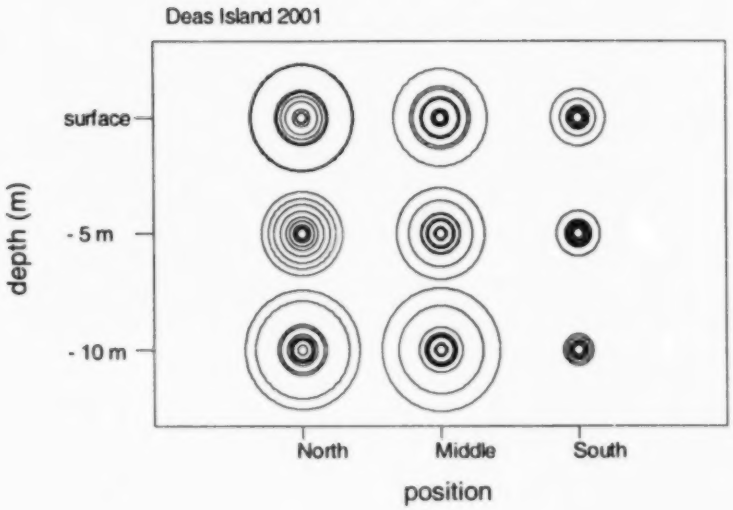
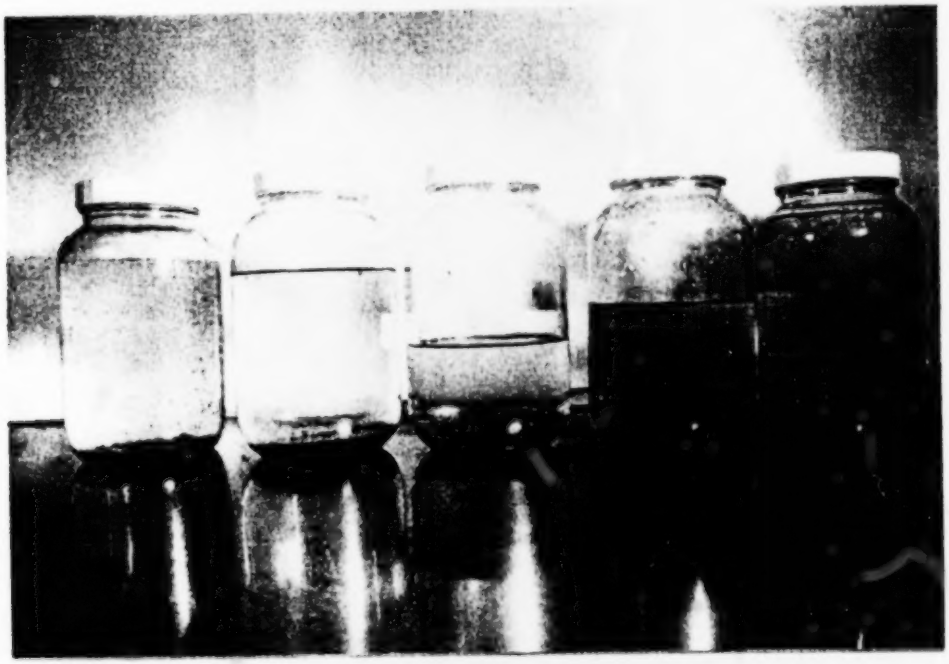


Fig. 7. Five typical Fraser River 1 litre sampling jars containing eulachon eggs, larvae and varying amounts of sand, silt and debris. Eggs and larvae are sieved using 2 mm and 600 micron aperture stainless steel sieves.



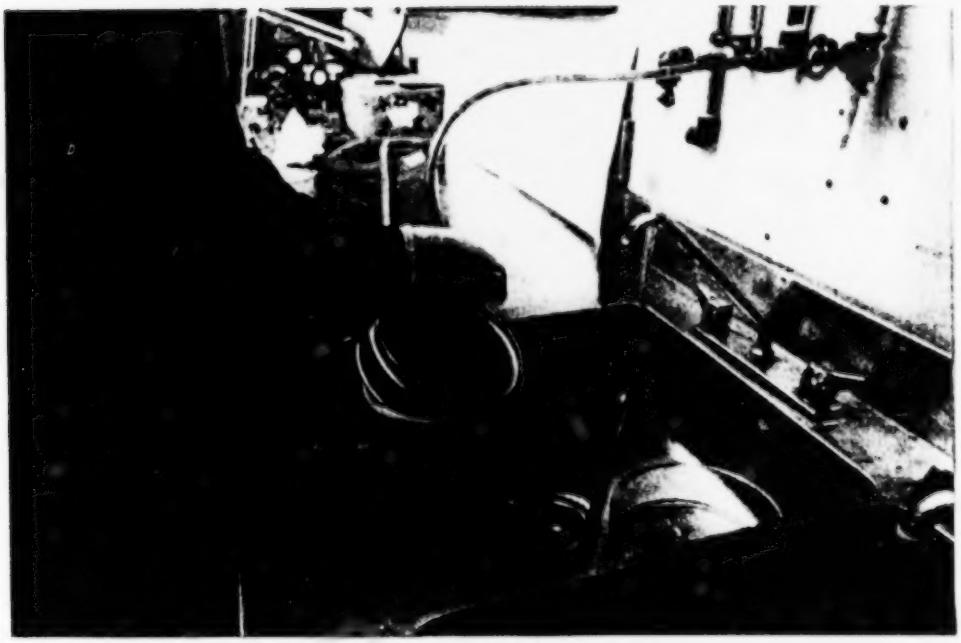


Fig. 8. Larval eulachon density map of the Douglas Channel Region (DC) during May 27 – June 7, 1996 (Maximum density = 32.2 larvae/m3). A cross indicates a station where no eulachon larvae were captured.

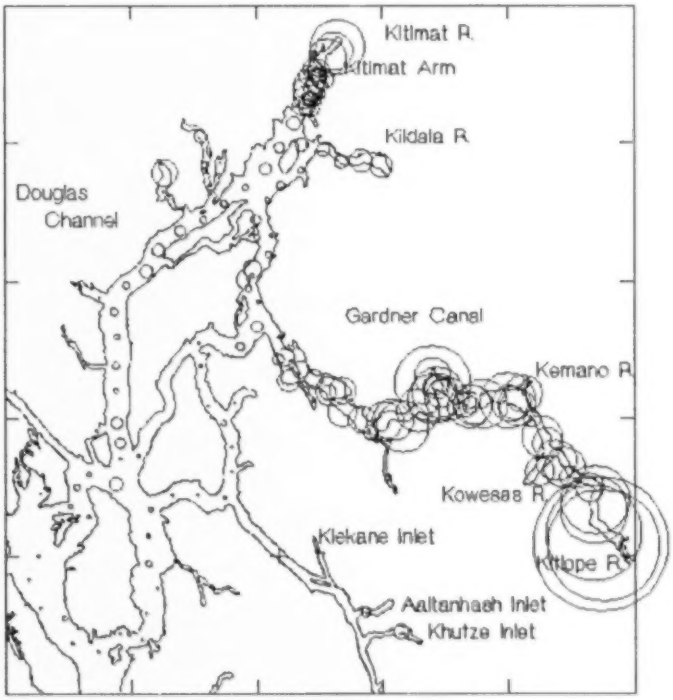


Fig. 9. Larval eulachon density map of the Johnstone Strait Region (JS) during April 14 – 25, 1997 (Maximum density = 6.5 larvae/m3). A cross indicates a station where no eulachon larvae were captured.

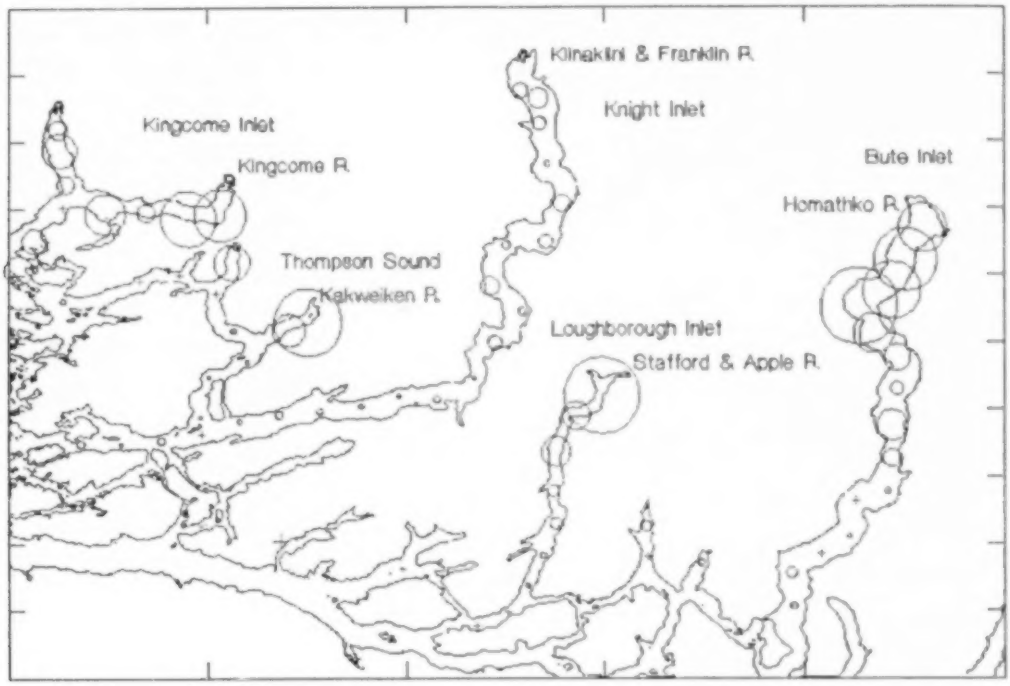


Fig. 10. Larval eulachon density map of the Rivers Inlet Region (RI) during April 14 – 25, 1997 (Maximum density = 3.6 larvae/m3). A cross indicates a station where no eulachon larvae were captured.

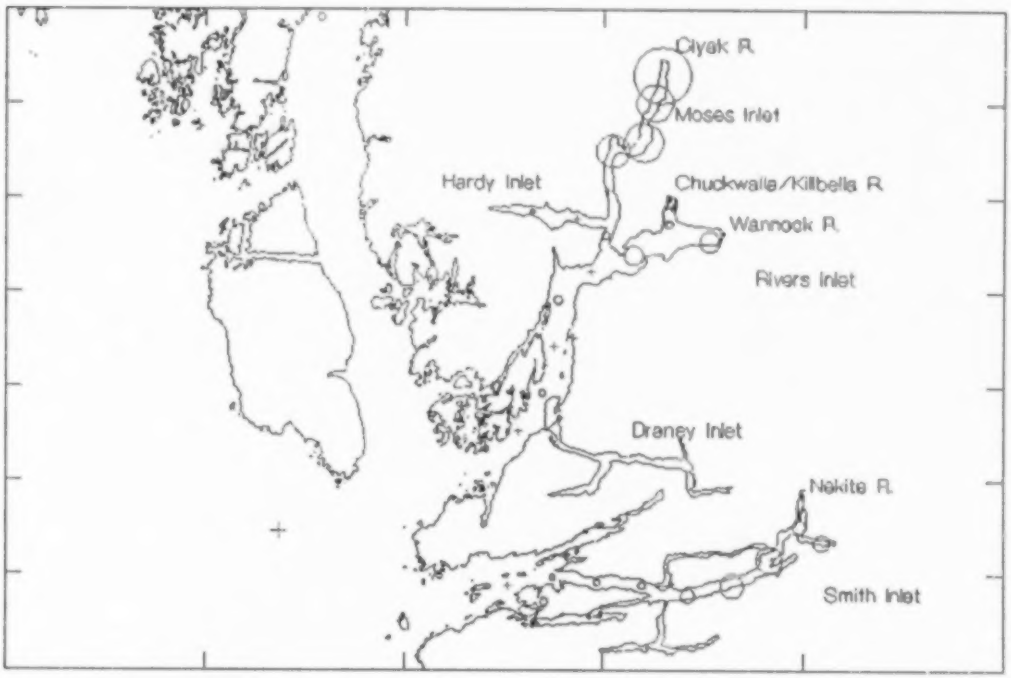


Fig. 11. Larval eulachon density map of the Burke and Dean Channel Region (BD) during April 14 – 25, 1997 (Maximum density = 1.4 larvae/m3). A cross indicates a station where no eulachon larvae were captured.

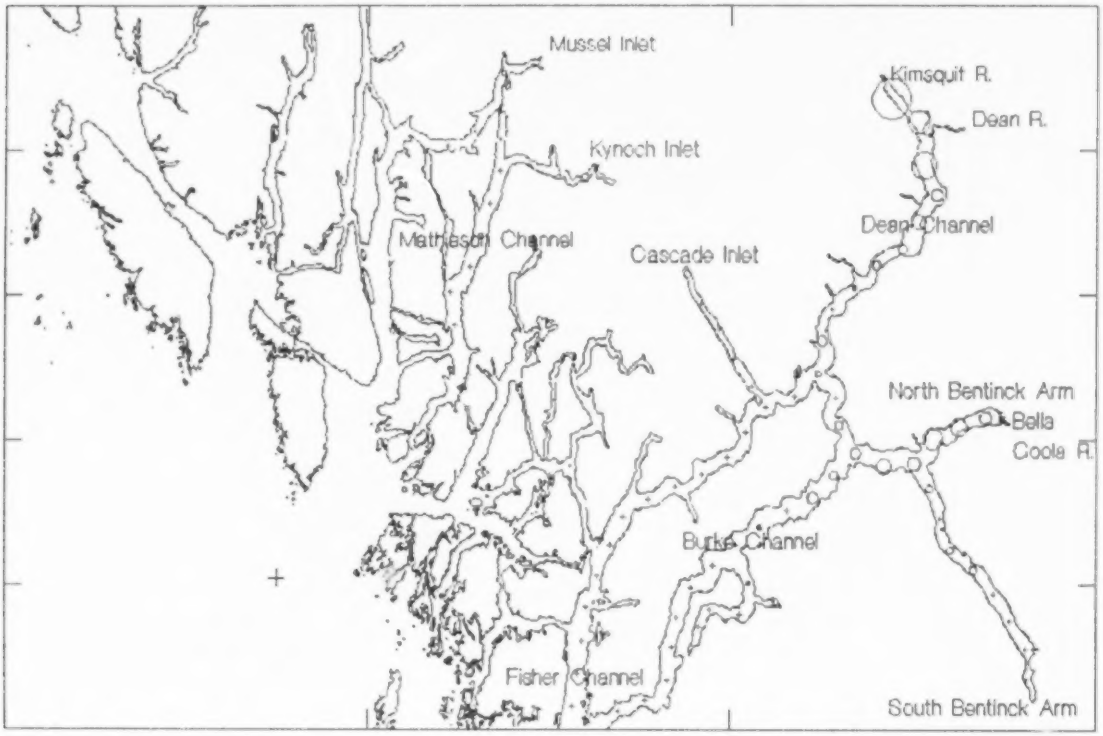


Fig. 12. Larval eulachon mean lengths (N=20) are represented by the size of a triangle at each sampling station along Gardner Canal (May 27 – June 7, 1996). Mean lengths ranged from 5.2 mm at the head of Gardner Canal near the Kitlope River estuary to 12.1 mm where Gardner Canal joins Douglas Channel. A small dot or cross indicates a station where less than 20 eulachon larvae were captured.